

**Current Status of the Claims:**

This listing of claims will replace the listing of claims in the application:

**Listing of Claims:**

1. (Original) An isolated infectious recombinant respiratory syncytial virus (RSV) comprising a recombinant RSV genome or antigenome, a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase protein (L), and a RNA polymerase elongation factor, wherein the recombinant genome or antigenome incorporates a heterologous polynucleotide encoding an immune modulatory molecule.
2. (Original) The recombinant RSV of claim 1, wherein the immune modulatory molecule is a cytokine, chemokine, enzyme, cytokine antagonist, chemokine antagonist, surface receptor, soluble receptor, adhesion molecule, or ligand.
3. (Original) The recombinant RSV of claim 2, wherein the immune modulatory molecule is a cytokine selected from interleukin 2 (IL-2), interleukin 4 (IL-4), interferon gamma (IFN $\lambda$ ), or granulocyte-macrophage colony stimulating factor (GM-CSF).
4. (Original) The recombinant RSV of claim 2, wherein the cytokine is interferon gamma (IFN $\lambda$ ).
5. (Original) The recombinant RSV of claim 2, wherein the cytokine is granulocyte-macrophage colony stimulating factor (GM-CSF).
6. (Original) The recombinant RSV of claim 2, wherein the cytokine is interleukin 2 (IL-2).
7. (Original) The recombinant RSV of claim 2, wherein the cytokine is interleukin 4 (IL-4).
8. (Original) The recombinant RSV of claim 1, wherein introduction of the cytokine into the recombinant genome or antigenome confers one or more desired phenotypic changes in the recombinant RSV compared to a wild type or mutant parental

virus comprising (i) a change in viral growth in cell culture, (ii) attenuation in the lower and/or upper respiratory tract(s) of a mammalian host; (iii) a change in plaque size, or (iv) a change in cytopathogenicity or confers one or more altered host immune response(s) selected from an anti-RSV neutralizing antibody response, a T-helper cell response, a cytotoxic T cell (CTL) response, and/or a natural killer (NK) cell response compared to a host immune response(s) elicited by the wild type or mutant parental virus.

9. (Original) The recombinant RSV of claim 1, wherein viral growth in cell culture is attenuated by approximately 10-15 fold or greater compared to growth of the corresponding wild-type or mutant parental RSV strain.

10. (Original) The recombinant RSV of claim 1, wherein the recombinant virus expresses the immune modulatory molecule in cell culture medium at a level of between about 10-20 micrograms per  $10^6$  cells or greater.

11. (Original) The recombinant RSV of claim 1, wherein the virus is attenuated for growth in cell culture and for replication in both the upper and lower respiratory tracts of a mammalian host, and wherein the virus elicits a protective immune response to RSV in a vaccinated host.

12. (Original) The recombinant RSV of claim 1, wherein the virus is attenuated by the activity of the immune modulatory molecule expressed in infected cells.

13. (Original) The recombinant RSV of claim 1, wherein the virus induces titers of serum IgG that are 2 to 10-fold higher or more than levels of serum IgG induced by wild type RSV.

14. (Original) The recombinant RSV of claim 1, wherein the genome or antigenome is further modified by introduction of one or more attenuating mutations identified in a biologically derived mutant human RSV.

15. (Original) The recombinant RSV of claim 14, wherein the genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present within a panel of biologically derived mutant human RSV strains, said

panel comprising cpts RSV 248 9ATCC VR 2450), cpts RSV 248/404 (ATCC VR 2454), cpts RSV 248/955 (ATCC VR 2453), cpts RSV 530 (ATCC VR 2452), cpts RSV 530/1009 (ATCC VR 2451), cpts RSV 530/1030 (ATCC VR 2455), RSV B-1 cp52/2B5 (ATCC VR 2542), and RSV B-1 cp-23 (ATCC VR 2579).

16. (Original) The recombinant RSV of claim 14, wherein the recombinant genome or antigenome incorporates at least one and up to a full complement of attenuating mutations specifying an amino acid substitution at Val267 in the RSV N gene, Glu218 and/or Thr523 in the RSV F gene, Asn43, Cys319, Phe 521, Gln831, Met1169, Tyr1321 and/or His 1690 in the RSV polymerase gene L, and a nucleotide substitution in the gene-start sequence of gene M2.

17. (Original) The recombinant RSV of claim 14, wherein the genome or antigenome incorporates at least two attenuating mutations.

18. (Original) The recombinant RSV of claim 14, wherein the genome or antigenome includes at least one attenuating mutation stabilized by multiple nucleotide changes in a codon specifying the mutation.

19. (Original) The recombinant RSV of claim 1, wherein the genome or antigenome comprises an additional nucleotide modification specifying a phenotypic change selected from a change in growth in cell culture, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, antigen expression, or a change in immunogenicity.

20. (Original) The recombinant RSV of claim 19, wherein the additional nucleotide modification alters a SH, NS1, NS2, M2 ORF2, or G gene of the recombinant RSV.

21. (Original) The recombinant RSV of claim 20, wherein a SH, NS1, NS2, M2 ORF2, or G gene is deleted in whole or in part or expression of the gene is reduced or ablated by a frame shift or introduction of one or more stop codons in an open reading frame of the gene or a modification of a translational start site.

22. (Original) The recombinant RSV of claim 19, wherein the additional nucleotide modification comprises a nucleotide deletion, insertion, substitution, addition or rearrangement of a cis-acting regulatory sequence of a selected gene within the recombinant RSV genome or antigenome.

23. (Original) The recombinant RSV of claim 19, wherein a gene end (GE) signal of the NS1 or NS2 gene is modified.

24. (Original) The recombinant RSV of claim 19, wherein the additional nucleotide modification comprises an insertion, deletion, substitution, or rearrangement of a translational start site within the recombinant RSV genome or antigenome.

25. (Original) The recombinant RSV of claim 24, wherein the translational start site for a secreted form of the RSV G glycoprotein is ablated.

26. (Original) The recombinant RSV of claim 19, wherein the genome or antigenome is modified to encode a non-RSV molecule selected from one or more T-helper epitope(s), a restriction site marker, or a protein of a microbial pathogen capable of eliciting a protective immune response in a mammalian host.

27. (Original) The recombinant RSV of claim 19, wherein the genome or antigenome incorporates a gene or genome segment from parainfluenza virus (PIV).

28. (Original) The recombinant RSV of claim 27, wherein the gene or genome segment encodes a PIV HN or F glycoprotein or immunogenic domain or epitope thereof.

29. (Original) The recombinant RSV of claim 27, wherein the genome segment encodes an ectodomain or immunogenic epitope of HN or F of PIV1, PIV2, or PIV3.

30. (Original) The recombinant RSV of claim 1, wherein the genome or antigenome comprises a partial or complete RSV background genome or antigenome of a human or bovine RSV combined with a heterologous gene or genome segment of a different RSV to form a human-bovine chimeric RSV genome or antigenome.

31. (Original) The recombinant RSV of claim 30, wherein the heterologous gene or genome segment encodes a RSV F, G or SH glycoprotein or an immunogenic domain or epitope thereof.

32. (Original) The recombinant RSV of claim 30, wherein the heterologous gene or genome segment is substituted for a counterpart gene or genome segment in a partial RSV background genome or antigenome

33. (Original) The recombinant RSV of claim 30, wherein the heterologous gene or genome segment is added adjacent to or within a noncoding region of the partial or complete RSV background genome or antigenome

34. (Original) The recombinant RSV of claim 30, wherein the chimeric genome or antigenome comprises a partial or complete human RSV background genome or antigenome combined with a heterologous gene or genome segment from a bovine RSV

35. (Original) The recombinant RSV of claim 30, wherein the chimeric genome or antigenome comprises a partial or complete bovine RSV background genome or antigenome combined with a heterologous gene or genome segment from a human RSV

36. (Original) The recombinant RSV of claim 34, wherein one or more human RSV glycoprotein genes F, G and SH or a genome segment encoding a cytoplasmic domain, transmembrane domain, ectodomain or immunogenic epitope thereof is substituted for a counterpart gene or genome segment within the bovine RSV background genome or antigenome

37. (Original) The recombinant RSV of claim 36, wherein one or both human RSV glycoprotein genes F and G is substituted to replace one or both counterpart F and G glycoprotein genes in the bovine RSV background genome or antigenome.

38. (Original) The recombinant RSV of claim 37, wherein both human RSV glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein genes in the bovine RSV background genome or antigenome.

39. (Original) The recombinant RSV of claim 36, wherein the heterologous gene or genome segment is from a subgroup A or subgroup B human RSV.

40. (Original) The recombinant RSV of claim 36, wherein the human-bovine chimeric genome or antigenome incorporates antigenic determinants from both subgroup A and subgroup B human RSV.

41. (Original) The recombinant RSV of claim 1 which is a virus.

42. (Original) The recombinant RSV of claim 1 which is a subviral particle.

43. (Original) A method for stimulating the immune system of an individual to induce protection against RSV which comprises administering to the individual an immunologically sufficient amount of the recombinant RSV of claim 1 combined with a physiologically acceptable carrier.

44. (Original) The method of claim 43, wherein the recombinant RSV is administered in a dose of  $10^3$  to  $10^7$  PFU.

45. (Original) The method of claim 43, wherein the recombinant RSV is administered to the upper respiratory tract.

46. (Original) The method of claim 43, wherein the recombinant RSV is administered by spray, droplet or aerosol.

47. (Original) The method of claim 43, wherein the recombinant RSV is administered to an individual seronegative for antibodies to RSV or possessing transplacentally acquired maternal antibodies to RSV.

48. (Original) The method of claim 43, wherein the recombinant RSV is attenuated and exhibits increased antigen expression compared to growth and antigen expression of a corresponding wild-type or mutant parental RSV strain.
49. (Original) The method of claim 43, wherein the recombinant RSV elicits an immune response against human RSV A, human RSV B, or both.
50. (Original) An immunogenic composition to elicit an immune response against RSV comprising an immunologically sufficient amount of the recombinant RSV of claim 1 in a physiologically acceptable carrier.
51. (Original) The immunogenic composition of claim 50, formulated in a dose of  $10^3$  to  $10^7$  PFU.
52. (Original) The immunogenic composition of claim 50, formulated for administration to the upper respiratory tract by spray, droplet or aerosol.
53. (Original) The immunogenic composition of claim 50, wherein the recombinant RSV exhibits attenuated growth and increased antigen expression compared to growth and antigen expression of a corresponding wild-type or mutant parental RSV strain.
54. (Original) The immunogenic composition of claim 50 which elicits an immune response against human RSV A, human RSV B, or both.
55. (Withdrawn) An isolated polynucleotide molecule comprising a RSV genome or antigenome which is modified to incorporate a polynucleotide sequence encoding an immune modulatory molecule.
56. (Withdrawn) The isolated polynucleotide molecule of claim 55, wherein immune regulatory molecule is a cytokine.
57. (Withdrawn) The isolated polynucleotide molecule of claim 56, wherein the cytokine is selected from interleukin 2 (IL-2), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL6), interleukin 18 (IL-18), tumor necrosis factor (TNF) alpha,

interferon gamma (IFN), or granulocyte-macrophage colony stimulating factor (GM-CSF)

58. (Withdrawn) The isolated polynucleotide molecule of claim 55, wherein the genome or antigenome is further modified by introduction of one or more attenuating mutations identified in a biologically derived mutant human RSV wherein both human RSV glycoprotein genes F and G are substituted to replace counterpart F and G glycoprotein genes in the bovine RSV genome or antigenome.

59. (Withdrawn) The isolated polynucleotide molecule of claim 55, wherein the genome or antigenome comprises an additional nucleotide modification specifying a phenotypic change selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

60. (Withdrawn) The isolated polynucleotide molecule of claim 59, wherein the genome or antigenome is modified by deletion of a SH, NS1, NS2, G gene or M2-2 ORF in whole or in part or by introduction of a frame shift or stop codon in an open reading frame of the gene that reduces or ablates gene expression.

61. (Withdrawn) The isolated polynucleotide molecule of claim 60, wherein a SH, NS1, NS2, or G gene or M2-2 ORF is deleted in whole or in part.

62. (Withdrawn) The isolated polynucleotide molecule of claim 59, wherein the nucleotide modification comprises a nucleotide deletion, insertion, addition or rearrangement of a cis-acting regulatory sequence of a selected RSV gene within the RSV genome or antigenome.

63. (Original) A method for producing an infectious attenuated RSV particle from one or more isolated polynucleotide molecules encoding said RSV, comprising:  
expressing in a cell or cell-free lysate an expression vector comprising an isolated polynucleotide comprising a recombinant RSV genome or antigenome which is modified to incorporate a polynucleotide sequence encoding an immune modulatory molecule, and RSV N, P, L and RNA polymerase elongation factor proteins.



64. (Original) The method of claim 63, wherein the recombinant RSV genome or antigenome and the N, P, L and RNA polymerase elongation factor proteins are expressed by two or more different expression vectors.

65. (Currently Amended) ~~The isolated infectious recombinant RSV of claim 1~~ method of claim 63, wherein the recombinant genome or antigenome comprises a partial or complete RSV vector genome or antigenome combined with one or more heterologous genes or genome segments encoding one or more antigenic determinants of one or more heterologous pathogens.

66. (Currently Amended) ~~The isolated infectious recombinant RSV~~ method of claim 65, wherein said one or more heterologous pathogens is a heterologous RSV and said heterologous gene(s) or genome segment(s) encode(s) one or more RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2), L, F or G protein(s) or fragment(s) thereof.

67. (Currently Amended) ~~The isolated infectious recombinant RSV~~ method of claim 65, wherein the vector genome or antigenome is a partial or complete RSV A genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of a RSV B subgroup virus.

68. (Currently Amended) The isolated infectious recombinant RSV of claim 65, wherein the chimeric genome or antigenome incorporates one or more gene(s) or genome segment(s) of a BRSV that specifies attenuation.

69. (Currently Amended) ~~The isolated infectious recombinant RSV~~ method of claim 65, wherein one or more HPIV1, HPIV2, or HPIV3 gene(s) or genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or complete HRSV vector genome or antigenome.

70. (Currently Amended) ~~The isolated infectious recombinant RSV~~ method of claim 65, wherein the vector genome or antigenome is a partial or complete BRSV genome or antigenome and the heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more HRSV(s).

71. (Currently Amended) The ~~isolated infectious recombinant RSV~~ method of claim 70, wherein the partial or complete BRSV genome or antigenome incorporates one or more gene(s) or genome segment(s) encoding one or more HRSV glycoprotein genes selected from F, G and SH, or one or more genome segment(s) encoding cytoplasmic domain, transmembrane domain, ectodomain or immunogenic epitope portion(s) of F, G, and/or SH of HRSV.

72. (Currently Amended) The ~~isolated infectious recombinant RSV~~ method of claim 65, wherein the vector genome or antigenome is a partial or complete HRSV or BRSV genome or antigenome and the heterologous pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

73. (Currently Amended) The ~~isolated infectious recombinant RSV~~ method of claim 72, wherein said one or more heterologous antigenic determinant(s) is/are selected from measles virus HA and F proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and NS1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes thereof.

74. (Currently Amended) The ~~isolated infectious recombinant RSV~~ method of claim 72, wherein the heterologous pathogen is measles virus and the heterologous antigenic determinant(s) is/are selected from the measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

75. (Currently Amended) The isolated infectious recombinant RSV of claim 74, wherein a transcription unit comprising an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within a HRSV vector genome or antigenome.

76. (Currently Amended) The ~~isolated-infectious-recombinant RSV~~ method of claim 1, wherein the recombinant genome or antigenome has one or more shifted RSV gene(s) or genome segment(s) within said recombinant genome or antigenome that is/are positionally shifted to a more promoter-proximal or promoter-distal position relative to a position of said RSV gene(s) or genome segment(s) within a wild type RSV genome or antigenome.

77. (Currently Amended) The ~~isolated-infectious-recombinant RSV~~ method of claim 76, wherein said one or more shifted gene(s) or genome segment(s) is/are shifted to a more promoter-proximal or promoter-distal position by deletion, insertion or rearrangement of one or more displacement polynucleotide(s) within said partial or complete recombinant RSV genome or antigenome.

78. (Currently Amended) The ~~isolated-infectious-recombinant RSV~~ method of claim 77, wherein said displacement polynucleotide(s) comprise(s) one or more polynucleotide insert(s) of between 150 nucleotides (nts) and 4,000 nucleotides in length which is inserted in a non-coding region (NCR) of the genome or antigenome or as a separate gene unit (GU), said polynucleotide insert lacking a complete open reading frame (ORF) and specifying an attenuated phenotype in said recombinant RSV.

79. (Currently Amended) The ~~isolated-infectious-recombinant RSV~~ method of claim 76, wherein said displacement polynucleotide(s) comprise(s) one or more RSV gene(s) or genome segment(s) selected from RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2), L, F and G genes and genome segments and leader, trailer and intergenic regions of the RSV genome and segments thereof.

80. (Currently Amended) The ~~isolated-infectious-recombinant RSV~~ method of claim 76, wherein said displacement polynucleotide(s) comprise(s) one or more bovine RSV (BRSV) or human RSV (HRSV) gene(s) or genome segment(s) selected from RSV NS1, NS2, N, P, M, SH, M2(ORF1), M2(ORF2), L, F and G gene(s) or genome segment(s) and leader, trailer and intergenic regions of the RSV genome or segments thereof.

81. (Currently Amended) The ~~isolated-infectious-recombinant RSV method~~ of claim 80, wherein said displacement polynucleotide(s) is/are deleted to form the recombinant RSV genome or antigenome to cause a positional shift of said one or more shifted RSV gene(s) or genome segment(s) within said recombinant genome or antigenome to a more promoter-proximal position relative to a position of said RSV gene(s) or genome segment(s) within a wild type RSV genome or antigenome.

82. (Currently Amended) The ~~isolated-infectious-recombinant RSV method~~ of claim 81, wherein said displacement polynucleotide(s) that is/are deleted to form the recombinant RSV genome or antigenome comprise one or more RSV NS1, NS2, SH, M2(ORF2), or G gene(s) or genome segment(s) thereof.

83. (Currently Amended) The ~~isolated-infectious-recombinant RSV method~~ of claim 76, wherein the RSV glycoprotein gene G is rearranged within said recombinant RSV genome or antigenome to a gene order position that is more promoter-proximal compared to the wild type gene order position of G.

84. (Currently Amended) The ~~isolated-infectious-recombinant RSV method~~ of claim 76, wherein the RSV glycoprotein gene F is rearranged within said recombinant RSV genome or antigenome to a gene order position that is more promoter-proximal compared to the wild type gene order position of F.

85. (Currently Amended) The ~~isolated-infectious-recombinant RSV method~~ of claim 76, wherein both RSV glycoprotein genes G and F are rearranged within said recombinant RSV genome or antigenome to gene order positions that are more promoter-proximal compared to the wild type gene order positions of G and F.

86. (Currently Amended) The ~~isolated-infectious-recombinant RSV method~~ of claim 85, wherein the RSV glycoprotein gene G is shifted to gene order position 1 and the RSV glycoprotein gene F is shifted to gene order position 2 within said recombinant RSV genome or antigenome.

87. (Currently Amended) The ~~isolated infectious recombinant RSV~~ method of claim 76, wherein the RSV SH and NS2 genes are both deleted to form the recombinant RSV genome or antigenome or antigenome.

88. (Currently Amended) The ~~isolated infectious recombinant RSV~~ method of claim 76, wherein the RSV SH, NS1 and NS2 genes are all deleted to form the recombinant RSV genome or antigenome or antigenome.